

REMARKS/ARGUMENTS

With this amendment, claims 37-51 are pending. New claims 49-51 are added. For convenience, the Examiner's rejections are addressed in the order presented in a May 6, 2005, Office Action. Applicants thank Examiner Rao for his time in meeting with inventor Dr. Warren Wakarchuk and Applicants' representative, Beth Kelly during an in person interview on August 30, 2005. The rejections under 35 U.S.C. §103(a) were discussed, but no agreement was reached.

I. Status of the claims

Claims 45, 47, and 48 are amended to correct typographical errors. These amendments are not limiting amendments and add no new matter.

New claim 49 recites that the CMP-Neu5Ac synthetase is encoded by a nucleic acid that is amplified by a first primer of SEQ ID NO:3 and a second primer of SEQ ID NO:4. Support for this amendment is found throughout the specification, for example at page 39 lines 31 through page 40, line 5. New claim 50 depends from claim 37 and recites that the α -2,3-sialyltransferase is encoded by a nucleic acid that is amplified by a first primer of SEQ ID NO:5 and a second primer of SEQ ID NO:6. New claim 51 depends from claim 49 and also recites that the α -2,3-sialyltransferase is encoded by a nucleic acid that is amplified by a first primer of SEQ ID NO:5 and a second primer of SEQ ID NO:6. These amendments add no new matter.

II. Rejections under 35 U.S.C. §103(a)

Claims 37-48 are rejected under 35 U.S.C. §103(a) as being allegedly obvious in view of Bulow *et al.*, *TIBtech* 9:226-231 (1991); Defrees *et al.*, WO 96/32491; and the common knowledge of the art of molecular biology provided by Sambrook *et al.*, pages 7.37-7.52 (1989) in further view of Gilbert(a) *et al. Eur. J. Biochem.*, 249:187-194 (1997) and Gilbert(b) *et al. Biotech. Lett.* 19:417-420 (1997). Applicants respectfully traverse the rejection.

To establish a *prima facie* case of obviousness, three basic criteria must be met:
(1) there must be some suggestion or motivation, either in the references themselves or in the

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knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings; (2) there must be a reasonable expectation of success; and (3) the prior art reference must teach or suggest all the claims limitations. MPEP§2143.

Applicants continue to assert that the cited references, alone or in combination, fail to teach or suggest all the elements of the claimed invention, fail to teach or suggest their combination to arrive at the claimed invention, and fail to provide a reasonable expectation of success in the combination. Thus, Applicants continue to assert that a prima facie case of obviousness has not been provided in this Advisory Action or any previous Office Action.

A. *Bulow et al. does not provide motivation for fusion of a glycosyltransferase and an accessory enzyme.*

According to the Office Action, Bulow *et al.* provide a motivation or suggestion for the fusion of two enzymes saying that the close proximity of the two enzymes will result in favorable enzyme kinetics for the coupled reaction. Applicants submit as Exhibit A, a declaration from one of skill in the art, Dr. James Paulson, in support of non-obviousness of the invention. Dr. Paulson reviewed the Bulow *et al.* reference relied on by the Office Action and discusses the reference.

Dr. Paulson explains that at the time of filing the proximity effect disclosed by Bulow *et al.* had been discredited. Dr. Paulson reviewed work on the proximity effect between publication of Bulow *et al.* in 1991 and the filing of the priority application in December 1997. First, researchers established that the proximity effect cannot be explained by simple diffusion between active sites on the same protein and proposed the existence of electrostatic regions on the surface of the fusion protein that allowed substrate channeling between the active sites of the enzyme components. *See, e.g.,* Elcock and McCammon, *Biochemistry* 35:12652-12658 (1996); submitted herein as Exhibit C.

Researchers then began to doubt the substrate channeling proximity effect. Naturally-occurring bi-functional fusion proteins that supposedly exhibit the proximity effect had been identified and their structure had been solved. *See, e.g.,* Trujillo *et al. Prot. Eng.* 10:567-

573 (1997); submitted herein as Exhibit D. Using the structure of a naturally occurring bi-functional fusion protein as a model, Trujillo *et al.* constructed a synthetic bi-functional fusion protein with mono-functional proteins from a different organism. However, even with kinetic and structural information of the naturally occurring bi-functional enzyme available, Trujillo *et al.* were unable to demonstrate a proximity effect in the synthetic bi-functional fusion protein. Thus, after Bulow *et al.* was published, researchers first found it necessary to revise the proximity effect theory to fit new data, and then found that the proximity effect theory could not be verified, even under the most optimal conditions, *i.e.*, with structural information supposed to allow construction of a bi-functional protein that exhibits the proximity effect. Moreover, according to Dr. Paulson, the proximity effect appears not to be evolutionarily conserved, as the naturally occurring bi-functional proteins believed to exhibit the proximity effect are found in other organisms as two separate mono-functional proteins. Thus, at the time of filing, based on additional analysis of the proximity effect in *e.g.*, Elcock and McCammon, and Trujillo *et al.*, those of skill would not have been motivated to combine the cited references to arrive a -bi-functional fusion protein comprising an accessory enzyme and a glycosyltransferase, and would not have had an expectation of success in doing so.

Trujillo *et al.* was published in May 1997, well before the December 12, 1997 priority date. According to Dr. Paulson more papers have been published discrediting the proximity effect, including a paper listing Leif Bulow of Bulow *et al.* as an author. *See, e.g.*, Pettersson *et al. Eur. J. Biochem.* 267:5041-5046 (2000), submitted herein as Exhibit E.

Based on the declaration of Dr. Paulson, the collection of cited references fails to provide a motivation for their combination to arrive at the claimed invention. In particular, without enhanced kinetics disclosed in Bulow *et al.*, the reference fails to provide motivation for or a reasonable expectation of success in arrival at a bi-functional fusion protein comprising a glycosyltransferase and a sialyltransferase, such as, *e.g.*, the claimed CMP-sialic acid and sialyltransferase.

B. Improved activity of the CMP-sialic acid/sialyltransferase fusion protein cannot be explained by the proximity effect of Bulow et al.

In the last response, Applicants asserted that Bulow *et al.* disclose improvements only in the activity of coupled reactions and not in the activity of the individual reaction components, as is disclosed in the application. The present Office Action continues to maintain that the invention relies on the proximity effect as taught by Bulow *et al.* and that Applicants arguments regarding the difference between coupled and uncoupled reactions is "highly misplaced." Office Action at pages 6-7. Applicants continue to assert that the improvements in the activity of the CMP-sialic acid/sialyltransferase fusion protein cannot be explained by the proximity effect of Bulow *et al.* The proximity effect is determined in a coupled assay only and depends on interaction between the activities of the fusion protein components, *i.e.*, an increased local concentration of an intermediate synthesized by the first enzyme (simultaneously the product of the first enzyme and substrate of the second enzyme) promotes enhanced activity of the second enzyme. Applicants reassert that the disclosed improvements are not explained by the proximity effect and below highlight the differences between a coupled enzymatic assay and enzymatic assays of the individual components of the fusion protein.

The improved kinetic activity of the proximity effect of Bulow *et al.* is based on a coupled reaction using sequential enzymes. For the sake of argument only, a CMP-sialic acid synthetase/sialyltransferase coupled reaction is exemplified. The coupled reaction conditions are found in the application at page 41, lines 24-29. In a coupled reaction, the two enzymes are added to a reaction mixture that contains exogenously added substrates, *e.g.*, CTP, sialic acid, and LacNAc-FEX, an acceptor of sialic acid. Please note, the reaction mixture has no CMP-sialic acid, a substrate that is required for activity of the sialyltransferase. The CMP-sialic acid synthase enzymatically converts the CTP and sialic acid to CMP-sialic acid. Only after CMP-sialic acid is generated by the CMP-sialic acid synthetase, is the sialyltransferase able to transfer sialic acid from CMP-sialic acid to the LacNAc-FEX acceptor. Thus, the activity of the sialyltransferase is dependent on the activity of the CMP-sialic acid synthetase. The proximity effect theory supposes that high local concentrations of the synthesized CMP-sialic acid are

found around the fused proteins or are generated by electrostatic transfer of the CMP-sialic acid from *e.g.*, the active site of the CMP-sialic acid synthetase to the active site of the sialyltransferase. This high localized concentration of CMP-sialic acid is postulated to lead to enhanced activity of the bi-functional fusion protein in a coupled assay as compared to a coupled assay of unfused mono-functional proteins that must lack the high localized concentration of a CMP-sialic acid intermediate. Therefore, to measure the proximity effect, comparisons are made between fused or unfused enzymes in coupled reactions. See, *e.g.*, Trujillo *et al.* and Pettersson *et al.*

In contrast, Applicants compared activities of each component enzyme in the bi-functional fusion protein to the appropriate activity of an unfused mono-functional protein. Thus, for a sialyltransferase assay, Applicants used a reaction mixture that contained CMP-sialic acid and the LacNAc-FEX acceptor, both in excess. Assays of sialyltransferase activity were separately performed on the bi-functional CMP-sialic acid synthetase/sialyltransferase fusion protein and on the unfused sialyltransferase protein. Production of sialylated LacNAc-FEX was measured. Because the appropriate substrates, *i.e.*, CTP and sialic acid, are not present, the CMP-sialic acid synthetase is not active and CMP-sialic acid is not transferred from the active site of CMP-sialic acid synthetase to the active site of the sialyltransferase. Therefore, there is no possibility that a localized high concentration of the CMP-sialic acid intermediate, *i.e.*, a proximity effect, influences activity of the bi-functional fusion protein when only the sialyltransferase activity of the fusion protein is measured.

Applicants also compared CMP-sialic acid synthetase activities in the bi-functional CMP-sialic acid synthetase/sialyltransferase fusion protein and in the mono-functional CMP-sialic acid synthetase. To assay CMP-sialic acid synthetase activity Applicants used a reaction mixture that contained CTP and sialic acid, both in excess. Assays of CMP-sialic acid synthetase activity were separately performed on the bi-functional CMP-sialic acid synthetase/sialyltransferase fusion protein and on the mono-functional CMP-sialic acid synthetase. Production of CMP-sialic acid was measured. Because the acceptor, *i.e.*, LacNAc-FEX, is not present, the sialyltransferase is not active in the fusion protein. Therefore, there is no

possibility that a proximity effect influences activity of the bi-functional fusion protein when only the CMP-sialic acid synthetase activity of the fusion protein is measured.

The proximity effect disclosed in Bulow *et al.* requires a higher localized concentration of a coupled reaction intermediate to facilitate enhanced kinetics and transfer of the intermediate from one active site to another on a fusion protein. The fusion protein has improved turnover numbers for single enzyme, uncoupled reactions. The proximity effect cannot explain this result and therefore, cannot provide motivation for the fusion of the enzymes.

C. Warner used hindsight to review the inventor's experiments.

Applicants also provide remarks on an additional reference cited by the Office Action in obviousness arguments: Warner, *Nature Biotech.* 16:720-721 (1998). However, Warner is not prior art and cannot be used to support the rejection for alleged obviousness.

According to the Office Action the disclosure of Warner can be used to demonstrate that the results disclosed in the application are not unexpected in view of Bulow *et al.* Office Action at page 7. Applicants respectfully disagree with this position because Warner wrote his article with full knowledge of the inventors' result. Warner is, therefore, improperly cited in the Office Action.

The Office Action alleges that Warner uses the word proximity to refer to the proximity effect of Bulow *et al.*, *i.e.*, improvements in the kinetics of a coupled reaction. However, as discussed in Section B, the improvements in activity of the fusion protein were measured using uncoupled, single enzyme reactions and thus, could not be the result of a proximity of effect as described by Bulow *et al.* Moreover, as discussed in Section A and in Dr. Paulson's declaration, the proximity effect had been discredited by the time of filing. Warner describes the result as "fortuitous" meaning occurring by chance. Warner does not in any way suggest that the improved enzyme activities were expected.

In addition, Warner had the benefit of hindsight. Warner read the inventors' paper and then wrote the article cited by the Office Action. Thus, Warner's analysis depends on knowledge of the end result. Just as it is impermissible for an Examiner to use hindsight, it is

impermissible for the Examiner to cite a reference that analyzes the claimed invention with the benefit of hindsight.

Finally, the Warner article accompanied publication of the inventors' discovery in *Nature Biotechnology*, one of the premier journals in biological/biochemical sciences. The inventor's ability to publish the result in one of the most respected peer-reviewed scientific journals demonstrates that the result was groundbreaking and noteworthy to the entire scientific community. Moreover, the inventors' article was selected to be accompanied by commentary, *i.e.*, the Warner article, further highlighting the result described for the scientific community. A routine and obvious result would not receive this attention from a prestigious peer-reviewed journal. Therefore, Warner has been erroneously used in the Office Action as evidence of an alleged pre-filing response by one of skill.

D. The specific activity values disclosed in Bulow et al. can be compared to the turnover numbers disclosed in the application.

Finally, in the last response Applicants pointed out that the Bulow *et al.* reference discloses that, when corrected for the increase in molecular weight, specific activity of a fusion protein component is between 50-100% of the activity of the unfused protein. Bulow *et al.* did not disclose an increase in specific activity or any other activity measure after enzyme fusion. In contrast, Applicants pointed out that the specification disclosed that both components of the claimed CMP-sialic acid synthetase/sialyltransferase fusion protein exhibit higher turnover numbers as compared to unfused enzymes.

In the present Office Action the Examiner alleges that an enzyme's turnover number and specific activity are different characteristics of a protein being based on U/ μ M or U/mg, respectively. Following this reasoning, the Office Action concludes that the Bulow *et al.* disclosure on specific activity of a fusion protein compared to its unfused components cannot be used to support arguments of unexpected results.

During an in-person interview on August 31, 2005, inventor Dr. Warren Wakarchuk and Examiner Rao reviewed the relationship between specific activity and turnover

number of an enzyme. For the record, Applicants resubmit a Table given to Examiner Rao during the interview as Exhibit F. The table demonstrates that 1) both turnover number and specific activities of the fused CMP-sialic acid synthase and the sialyltransferase were increased as compared to the values for the unfused enzymes, and 2) the increase in specific activity and turnover number for the fused versus unfused enzymes were the same: for the CMP-sialic acid synthase a 1.26 ratio fused/unfused or 126% activity compared to unfused, for the sialyltransferase a 2.22 ratio fused/unfused or 222% activity compared to unfused.

Bulow *et al.* disclosed only 50-100% activity, *i.e.*, no improvement, of a fused enzyme compared to an unfused enzyme. The claimed CMP-sialic acid synthase/sialyltransferase fusion protein exhibits 126% increase or 222% increase, both values greater than those predicted by Bulow *et al.* The other cited references do not correct the deficiencies of Bulow *et al.* or predict any increase in activity on fusion. Therefore, the kinetic properties of the claimed were not disclosed in Bulow *et al.* and are not obvious as alleged by the Office Action.

Applicants have provided evidence that at the time of filing, the proximity effect theory proposed as an advantage by Bulow *et al.* had been discredited and that the proximity effect did not explain the improved properties of the claimed invention. Without the improved kinetics suggested by the proximity effect, any other advantage alleged to be taught by Bulow *et al.* cannot provide motivation. Thus, Bulow *et al.* cannot provide a motivation to combine the references to arrive at the claimed invention and cannot provide a reasonable expectation of success in doing so. The other cited references do not correct the deficiencies of Bulow *et al.* The citation of the Warner reference was done using impermissible hindsight. Finally, Applicants have explained the relationship of specific activity and enzyme turnover number to allow appropriate comparison between the disclosure of Bulow *et al.* and the results in the application. In view of the evidence submitted with this response and the arguments above, Applicants respectfully request that the rejection for alleged obviousness be withdrawn.

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Amdt. dated March 9, 2006
Amendment under 37 CFR 1.116 Expedited Procedure
Examining Group 1652

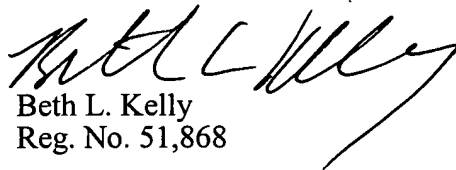
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CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance and an action to that end is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-576-0200.

Respectfully submitted,


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